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Linxian Nutrition Intervention Trials

Design, Methods, Participant Characteristics, and Compliance

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ABSTRACT

Two nutrition intervention trials were conducted in Linxian, China, where the esophageal/gastric cardia cancer mortality rates are among the highest in the world and there is suspicion that the population's chronic deficiencies of multiple nutrients are etiologically involved. Both trials were randomized, double-blind, and placebo-controlled, and tested the effect of multiple-vitamin and multiple-mineral supplements in lowering the rates of cancer. In the first trial, the Dysplasia Trial, 3318 individuals with a cytologic diagnosis of esophageal dysplasia received daily vitamin and mineral supplements or placebos for 6 years. The second trial, the General Population Trial, involved 29,584 individuals and used a one-half replicate of a 2^4 fractional factorial design, which enabled the testing of daily supplementation of four different vitamin and mineral combinations and placebo for a period 5 1/4 years. This article describes the design and methods of these studies as well as the baseline characteristics and compliance behavior of the participants in these two trials, the largest cancer chemoprevention studies reported to date. Ann Epidemiol 1993;3:577–585.

KEY WORDS: Esophageal cancer, gastric cardia cancer, stomach cancer, China, nutrition, trials, intervention, prevention, vitamins, minerals.

BACKGROUND

Some of the world's highest incidence and mortality rates for cancer of the esophagus occur in China (1, 2). There is dramatic geographic variation in the occurrence of esophageal cancer within China. The highest rates are found in Linxian, a rural county of approximately 800,000 residents, where the annual mortality rate from esophageal cancer exceeds national levels by nearly tenfold (3). The excess cancers occur not only as squamous cell carcinomas of the esophagus but also as adenocarcinomas of the gastric cardia. Traditionally, both tumors have been called "esophageal cancer" in Linxian, because of their proximity to one another and similarity in symptoms.

The reason for the exceptionally high cancer rates in Linxian is not known, but studies there over the past 30 years have identified several clues. Prominent are dietary hypotheses, including both the excessive ingestion of foods that may contain factors that increase risk (e.g., consumption of nitrosamine-contaminated fermented and moldy foods), and the inadequate consumption of foods that contain factors that may confer protection (e.g., riboflavin, retinol, alpha and beta carotene, ascorbic acid, alpha-tocopherol, zinc, and molybdenum) (4–7).

The high rates of cancer, low dietary intake of several nutrients, and administrative considerations favorable for implementation of a large trial combined to make Linxian a desirable setting in which to test a cancer chemoprevention strategy.

Two randomized, placebo-controlled trials were conducted. The first, the Dysplasia Trial, enrolled individuals with cytologically diagnosed esophageal dysplasia, while the second, the General Population Trial, enrolled individuals from the general population of the high-risk area (8). De-

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scribed here are the design, study methods, participant characteristics, and compliance for these two trials.

DYSPLASIA TRIAL

Objectives

The primary objective of this intervention was to evaluate the effect of multiple-vitamin and -mineral supplements on esophageal/gastric cardia cancer incidence and mortality in Linxian residents with a previous cytologic diagnosis of esophageal dysplasia. In addition, the effects of the supplements on mortality from other causes of death and on regression or progression of esophageal dysplasia were examined.

Study Design

Participants in the Dysplasia Trial were randomized to receive supplements (multivitamin and multimineral tablets and capsules) or matching placebos in a two-group design. Randomization was performed in blocks of ten within strata defined by commune, gender, and age. The daily dose and type of micronutrients in the supplements are shown in Table 1 and included a total of 14 vitamins and 12 minerals in doses typically two- to threefold, but ranging from 26 to 700%, the US recommended daily allowances (9). Each subject was given three pills daily, including one hard gelatin capsule (beta carotene as Solatene (Hoffmann-LaRoche) or placebo) and two tablets (Centrum (Lederle Laboratories) or placebos).

TABLE 1. Daily doses and types of micronutrients in the supplements in the Dysplasia Trial, Linxian, China[a]

Contains	As	Dose
Beta carotene		15 mg
Vitamin A	Acetate	10,000 IU
Vitamin E	*dl*-alpha-Tocopheryl acetate	60 IU
Vitamin C	Ascorbic acid	180 mg
Folic acid		800 μg
Vitamin B_1	Thiamine mononitrate	5 mg
Vitamin B_2	Riboflavin	5.2 mg
Niacinamide		40 mg
Vitamin B_6	Pyridoxine HCl	6 mg
Vitamin B_{12}	Cyanocobalamin	18 μg
Vitamin D		800 IU
Biotin		90 μg
Pantothenic acid	Calcium pantothenate	20 mg
Calcium	Dibasic calcium phosphate	324 mg
Phosphorus	Dibasic calcium phosphate	250 mg
Iodine	Potassium iodide	300 μg
Iron	Ferrous fumarate	54 mg
Magnesium	Magnesium oxide	200 mg
Copper	Cupric oxide	6 mg
Manganese	Manganese sulfate	15 mg
Potassium	Potassium chloride	15.4 mg
Chloride	Potassium chloride	14 mg
Chromium	Chromium chloride	30 μg
Molybdenum	Sodium molybdate	30 μg
Selenium	Sodium selenate	50 μg
Zinc	Zinc sulfate	45 mg

[a] Participants received two multivitamin, multimineral tablets (Centrum, Lederle Laboratories) and one beta carotene capsule (Solatene, Hoffmann-LaRoche) or matching placebos daily.

Eligibility Criteria and Exclusions

Potential participants were eligible if they were between the ages of 40 and 69 years, lived in one of three northern Linxian communes (Yaocun, Rencun, Donggang), provided consent, and had a diagnosis of esophageal dysplasia based on a balloon cytology examination. Individuals were excluded if they were taking any vitamin or mineral supplements regularly, had a history of malignancy or other debilitating disease, or were taking specific medications (i.e., retinoids or anti-tumor B, a traditional Chinese drug consisting of a mixture of six medicinal herbs). Table 2 shows the total number of participants in the Dysplasia Trial by eligibility, randomization, and exclusion status, and the final number in the analytic cohort.

Study Timeline

The diagnoses of esophageal dysplasia arose primarily from a population-based esophageal balloon cytology examination

TABLE 2. Total number of individuals in the nutrition intervention trials in Linxian, by eligibility, randomization, and exclusion status

	Dysplasia Trial	General Population Trial
Total potentially eligible individuals	3656	49,950
Nonparticipants		
Refusals	35	7992
Out of area	162	5994
Too sick	22	1499
Other	43	4182
Screened	3377	30,134
Randomized[a]	3394	30,283
Late exclusions[b]		
Cytology exclusion[c]	12	54
Self-reported cancer at screening	26	238
Cancer diagnosed before start of intervention	26	254
Died before start of intervention	12	153
Final analytic cohort	3318	29,584

[a] Individuals were randomized at the National Cancer Institute based on lists received from China. Only after receipt of all screening data was it learned that screening data were missing on 17 participants from the Dysplasia Trial and 149 participants from the General Population Trial.

[b] For the Dysplasia Trial, of the 76 late exclusions, 36 were in the placebo group and 40 in the supplement group. For the General Population Trial, of the 699 late exclusions, 94 were in the placebo group, 93 in AB, 76 in AC, 85 in AD, 91 in BC, 92 in BD, 87 in CD, and 81 in ABCD.

[c] For the Dysplasia Trial, 12 individuals (1 normal, 6 with hyperplasia, 3 with near-cancer, and 2 with cancer) did not have dysplasia based on the 1983 cytology examination and all of these were excluded. For the General Population Trial, 6873 individuals (6626 normal or with hyperplasia, 77 with dysplasia, 35 with near-cancer, 19 with cancer, and 116 unknown) took part in the 1983 cytology examination. The 54 with near-cancer or cancer were excluded.

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conducted in November and December 1983 in Yaocun, Rencun, and Donggang communes in Linxian, China (10). All residents of these three communes between the ages of 40 and 69 years were invited to participate, and approximately 13,000 individuals (37% of those eligible) underwent a balloon examination. This involved swallowing a deflated balloon covered with a mesh net. After passing into the stomach, the balloon was inflated and pulled up through the esophagus. Esophageal epithelial cells (and occasional epithelial cells from the gastric cardia) obtained in this way were examined using routine cytologic techniques. Individuals diagnosed with dysplasia 1 (low-grade dysplasia) or dysplasia 2 (high-grade dysplasia) by this or other recent balloon examinations totaled 3656 and all were invited to participate in the baseline screening phase of the intervention trial.

Baseline screening was performed between August and October 1984 and included interviews to obtain demographic, life-style, risk factor, and health status information; physical examinations; and blood and toenail sample collections. Nearly 3400 individuals (92% of those eligible) participated in the baseline screening and were randomized in November 1984. In January 1985, all 773 randomized individuals with a cytology diagnosis of dysplasia 2 were invited to participate in a pretrial endoscopy survey, and 520 (67%) underwent endoscopy. Pill delivery commenced at the beginning of May 1985 after a 6-month placebo run-in period conducted primarily to maintain subject interest until supplements became available. Although initially planned as a 5-year intervention, the trial was extended an additional 12 months, through the end of April 1991, a total of 72 months.

Special midtrial examinations were conducted from October to December 1987, after 30 months of intervention. A health interview, a brief physical examination, and a balloon swallow cytology examination were offered to all living trial participants and 2826 (88% of those eligible) took part. All Dysplasia Trial participants with a 1983 cytology diagnosis of dysplasia 2 and every fifth participant with a 1983 cytology diagnosis of dysplasia 1 were also invited to take part in an endoscopic survey. Sixty-three percent of those invited agreed and endoscopies were performed on 833 individuals.

In March to May 1991, end-of-trial examinations were conducted. All trial participants were invited for a health interview, a brief physical examination, a special ophthalmologic examination, a balloon swallow cytology examination, and blood and toenail sample collections. Of the 2765 participants alive and free of cancer at this time, 2398 (87%) were interviewed, 2413 (87%) had a physical examination, 1943 (70%) had a balloon swallow cytology examination, 2351 (85%) gave a blood sample, and 2366 (86%) provided toenail samples. In addition, 2141 subjects had ophthalmologic examinations. All participants from 19 of the 32 villages in Rencun commune who were under 70 years old, had no history of cancer, and had completed the cytologic examination were also invited to undergo endoscopy, and 396 individuals (81% of those invited) did so.

Participant Characteristics

Participant characteristics by treatment group are shown in Table 3. The median age of participants at the start of intervention in 1985 was 54, approximately one-fourth had dysplasia 2, and 43% reported a family history of either esophageal or stomach cancer in first-degree relatives. There were no statistically significant differences between the randomized groups with respect to any of the subject characteristics examined except for the grade of cytologic dysplasia from the 1983 balloon screening. Individuals randomized

TABLE 3. Dysplasia Trial participant characteristics by treatment group

Participant characteristics	No. on placebo (%)	No. on supplement (%)
No. of participants	1661	1657
Age (at beginning of intervention) (y)		
< 50	537 (32)	545 (33)
50–59	743 (45)	717 (43)
60+	381 (23)	395 (24)
Sex		
Male	731 (44)	730 (44)
Female	930 (56)	927 (56)
Cytology in 1983[a]		
Dysplasia 1	1302 (78)	1243 (75)
Dysplasia 2	359 (22)	414 (25)
Education		
None	685 (41)	719 (43)
Some	976 (59)	938 (57)
Tobacco (ever smoked cigarettes regularly > 6 mo)		
No	1184 (71)	1180 (71)
Yes	477 (29)	477 (29)
Alcohol[b] (any use in past 12 mo)		
No	1359 (82)	1328 (81)
Yes	294 (18)	321 (19)
Pickled vegetable (any use in past 12 mo in winter or spring)		
No	1492 (90)	1489 (90)
Yes	169 (10)	168 (10)
Moldy food[c] (any use in past 12 mo)		
No	1321 (80)	1333 (81)
Yes	322 (20)	316 (19)
Family history of esophageal cancer[d]		
No	992 (60)	984 (60)
Yes	661 (40)	665 (40)
Family history of stomach cancer[e]		
No	1602 (97)	1597 (97)
Yes	51 (3)	52 (3)

[a] P value = 0.02 for difference in distribution of dysplasia category by treatment group.
[b] Data missing on 16 participants (8 on placebo, 8 on supplement).
[c] Data missing on 35 participants (16 on placebo, 19 on supplement).
[d] Data missing on 16 participants (8 on placebo, 8 on supplement).
[e] Data missing on 16 participants (8 on placebo, 8 on supplement).

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to receive supplements had a 3% higher prevalence of dysplasia 2 in this examination than did those randomized to the placebo group.

Pill Delivery and Compliance Assessment

A team of over 200 village doctors delivered two bottles of pills monthly to each subject in the trial. At the time of the visit, bottles from the previous month were collected, residual pills were counted and recorded, new pill bottles were dispensed, and participants were asked about side effects. Specially developed mailers were used to mail pill bottles to participants who moved out of the area during the trial.

Compliance was assessed by counting unused pills monthly for all trial participants (except those who had moved from Linxian and received their pills by mail). As a quality-control measure, a team from the central field station periodically revisited a 10% sample of participants to check pill counts. Compliance was also assessed by serial cross-sectional nutritional assessment surveys performed quarterly on a sample of participants throughout the trial. For each quarterly survey, nine villages were randomly selected, followed by the random selection of 36 participants from these villages, apportioned within strata defined by age (< 50, 50 to 59, ≥ 60 years) and gender. For each survey, participants were asked to complete 24-hour dietary recall and food frequency questionnaires, and to provide blood samples. Blood was analyzed for retinol and beta carotene (11), for ascorbic acid (12), and for glutathione reductase activation coefficient as a measure of riboflavin status (13). Overall, 76% of participants selected took part in the nutritional assessment.

Compliance assessed by monthly pill counts indicated that among participants overall, 87% in the placebo and 89% in the supplement group took over 90% of their pills. Only 4% in each treatment group were poor compliers (i.e., took < 50% of pills). The overall pill disappearance rate was 94% in both groups, with only a slight decline (from 96% in year 1 to 92% in year 6 in both groups) over the duration of the trial. Results of pill recounts from the quality-control team indicated only slightly lower pill disappearance rates than those recorded by the village doctors.

Compliance assessed biochemically over the 6-year intervention is shown in Table 4. None of the nutrient levels differed between the randomized groups at baseline. In contrast, all values were statistically different in the supplement group (as compared with the placebo group) throughout the intervention period. Of the different nutrients assessed, beta carotene showed the greatest proportional change in blood levels of the treated individuals, with an average increase of more than tenfold over baseline values. Compared to baseline, values for riboflavin and ascorbic acid in participants in the placebo group improved during the intervention, suggesting a general improvement in nutritional status during the trial.

End Point Ascertainment

Incident cancers and deaths were identified through several methods that ensured essentially complete ascertainment of these events among trial participants. All medical facilities in the communes under study, plus the Linxian County Cancer Hospital and the cancer hospital in the prefecture capital of Anyang, provided rapid notice of all cancer diagnoses among residents of the communes in the trials. Participants with cancer symptoms and those who died from any cause were identified by village doctors on their monthly visits to deliver and retrieve pills. Additional visits to look for symptomatic individuals were made by a medical team from the Cancer Institute of the Chinese Academy of Medical Sciences (CICAMS) in Beijing based at a field station

TABLE 4. Dysplasia Trial compliance assessed biochemically over 6-year intervention

	Baseline[a]				During intervention				
	Placebo		Supplement		Placebo		Supplement		
Analyte	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	*P* value[b]
Retinol (μg/dL, plasma)	47	40.7 (14.4)	46	42.7 (13.6)	216	40.7 (13.4)	209	63.1 (17.6)	0.0001
Riboflavin (EGR AC)[c]	50	1.69 (0.27)	45	1.67 (0.29)	293	1.53 (0.35)	284	1.13 (0.23)	0.0001
Ascorbic acid (mg/dL, plasma)	45	0.60 (0.44)	43	0.62 (0.54)	283	0.63 (0.41)	281	0.92 (0.40)	0.0001
Beta carotene (μg/dL, plasma)	47	10.36 (5.2)	46	10.7 (6.1)	216	11.4 (15.9)	209	119.1 (86.8)	0.0001

[a] Baseline nutritional assessment conducted in September 1984; values were adjusted for season.
[b] *P* values are from *t* tests of difference between placebo and supplement during intervention.
[c] EGR activity measured in RBC hemolysates before and after addition of FAD. EGR AC is the ratio of glutathione reductase activity after the addition of FAD divided by glutathione reductase activity before the addition of FAD. An AC above 1.4 is considered to indicate deficient status; 1.20 to 1.40, marginal status; and less than 1.20, adequate status (6).
EGR = erythrocyte glutathione reductase; RBC = red blood cell; FAD = flavine adenine dinucleotide; AC = activity coefficient; SD = standard deviation.

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in Yaocun commune. Symptomatic individuals were referred to the CICAMS field station or their commune hospital for further evaluation. Cancers were also ascertained through the special cytology and endoscopy examinations conducted in 1987 and 1991. Attempts were made on all incident and fatal cancers to retrieve all relevant records and diagnostic materials (cytology slides, histology slides, and/or x-ray films) for later review. These records and materials were assembled and stored at the CICAMS field station.

Cancer cases with available diagnostic materials were reviewed by an International Endpoints Review Committee (IERC), composed of expert cytologists, pathologists, and radiologists from both the United States and China. The membership of the review committee is shown in the Appendix. Cancer cases without available diagnostic materials and all noncancer deaths were reviewed by a CICAMS Endpoints Review Committee composed of the Chinese members of the IERC. The review committees assigned a final diagnosis for each case, a site for each cancer case, and (when possible) a histologic type of cancer. They also recorded the basis for the cancer diagnoses (histology, cytology, x-ray film, and/or other) and assigned an end point designation (incident cancer, cancer death, noncancer death, or not an end point).

A total of 176 cancer deaths occurred during the Dysplasia Trial, including 82 due to esophageal cancer and 77 due to stomach cancer (66 from the gastric cardia). A total of 448 incident cancers, including 251 from the esophagus and 177 from the stomach (including 159 from the gastric cardia), were diagnosed during the Dysplasia Trial. The IERC reviewed 397 (89%) of these cancers. Of these 397 cases available for review, the diagnosis was based on histology in 69%, cytology in 45%, and x-ray films in 35%. Only one IERC case was based on clinical impression without other diagnostic materials.

Statistical Considerations

During the 6-year trial, the observed cumulative esophageal/gastric cardia cancer mortality was 4.5%, while the observed cumulative esophageal/gastric cardia cancer incidence was 12.4%. With 1659 participants in each arm and a two-sided test level of 0.05, power was 90% to detect a relative reduction of 51% in esophageal/gastric cardia cancer mortality from the intervention, and a relative reduction of 29% in incidence.

The primary methods of analysis used to analyze time-to-death information included calculation of rates and Cox proportional hazards regression models with incorporation of variables stratified on at randomization and risk factors from baseline found to be nonrandomly distributed. A lag period of 12 months following the start of intervention was incorporated in some analyses. For analyses of cancer incidence, which was affected by screenings, cumulative incidence was analyzed by logistic regression. The data were examined annually beginning in year 2 with the intention of stopping the trials only if some extreme level of statistical significance was reached (i.e., $P < 0.001$) (14). A three-person Data Safety and Monitoring Committee met periodically to review progress of the trial and make recommendations (see Appendix).

GENERAL POPULATION TRIAL

Objectives

The primary objective of this intervention was to evaluate the effect of multiple-vitamin and -mineral supplements on esophageal/gastric cardia cancer incidence and mortality in the general population of Linxian. A secondary objective was to examine the effect of these supplements on mortality from other causes of death in this population.

Study Design

For the General Population Trial a factorial design was chosen to provide more specificity than the two-group design (multiple micronutrients or placebo) used in the Dysplasia Trial. The trial enabled separate evaluation of four factors, designated by the letters A, B, C, and D. Daily doses and types of micronutrients by treatment factor are shown in Table 5. These doses are one to two times the US recommended daily allowances (9). While a separate evaluation of each of the nine, and perhaps additional, nutrients listed in Table 5 would have been desirable, a 2^9 or higher factorial experiment was impractical. The feasible options were to delete certain nutrients or to combine them into a smaller number of groups. We chose the latter, combining retinol with zinc (factor A), which enhances the delivery of retinol to tissues; the B vitamins riboflavin and niacin (factor B); vitamin C and molybdenum (factor C), which are both thought to inhibit the formation of carcinogenic nitrosamines; and the fat-soluble antioxidants beta carotene and

TABLE 5. Daily doses and types of micronutrients by treatment factor in the General Population Trial, Linxian, China

Factor	Micronutrients	Dose/d
A	Retinol (as palmitate)	5000 IU
	Zinc (as zinc oxide)	22.5 mg
B	Riboflavin	3.2 mg
	Niacin	40 mg
C	Ascorbic acid	120 mg
	Molybdenum (as molybdenum yeast complex)	30 μg
D	Beta carotene	15 mg
	Selenium (as selenium yeast)	50 μg
	Alpha-tocopherol	30 mg

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vitamin E with selenium (factor D). A fractional factorial design was selected because it permitted testing of the main effects of four factors at less cost and complexity than a full 2^4 factorial design (3).

Individuals were randomized to one of eight treatment groups corresponding to the following eight factor combinations: placebo, AB, AC, AD, BC, BD, CD, and ABCD. Thus, one-half of the participants received supplementation with each of the four vitamin and mineral combinations. For example, those in groups AB, AC, AD, and ABCD all received daily supplementation with retinol and zinc, while those in groups BC, BD, CD, and placebo did not. Randomization was performed in blocks of eight within strata defined by commune, gender, and age. Each subject was given two pills daily, including one hard gelatin capsule (beta carotene as Solatene (Hoffmann-LaRoche) or placebo) and one tablet (special preparation (Hoffmann-LaRoche) containing from zero to eight different micronutrients).

Eligibility Criteria and Exclusions

Individuals were eligible if they were between the ages of 40 and 69 years, lived in one of four northern Linxian communes (Yaocun, Rencun, Donggang, and Hengshui), and provided consent. Individuals were excluded if they were taking any vitamin or mineral supplements regularly, had a history of malignancy or other debilitating disease, or were taking specific medications (i.e., retinoids or anti-tumor B). Table 2 shows the total number of participants in the General Population Trial by eligibility, randomization, and exclusion status, and the final number in the analytic cohort.

Study Timeline

All adults between the ages of 40 and 69 years in Yaocun, Rencun, and Donggang communes in Linxian who were not already participating in the Dysplasia Trial were invited for baseline screening for the General Population Trial and 22,733 (65% of those eligible) took part. Because this number of individuals was less than required, all adults 40 to 69 years old from a fourth commune (Hengshui) were also invited. Overall, 30,134 individuals (61% of those eligible) took part in the baseline screening examinations for the General Population Trial conducted between March and May 1985. All forms and procedures used were identical to those of the Dysplasia Trial. Randomization occurred in July 1985. Intervention commenced at the beginning of March 1986 and continued through May 1991, a total of 63 months, 3 months more than initially planned.

In March to May 1991, at the end of the intervention, special end-of-trial examinations similar to those conducted in the Dysplasia Trial were performed. All trial participants were invited for a health interview, a brief physical examination, and a toenail sample collection. Of the 27,015 participants eligible (i.e., alive and cancer-free) at this time, 22,180 (82%) were interviewed, 21,319 (79%) had a physical examination, and 20,911 (77%) provided toenail samples. In addition, all trial participants from selected villages in Rencun commune were invited for a balloon swallow cytology examination and a blood sample collection. Of the 4402 participants eligible for these examinations, 3033 (69%) participated in the balloon swallow examination and 3872 (88%) provided blood samples. A special ophthalmologic examination was also conducted in 3249 participants.

Finally, all participants from two of the largest villages in Rencun who were under 70 years old, had no history of cancer, and had completed the cytologic examination were invited to undergo endoscopy, and 391 individuals (79% of those eligible) did so.

Participant Characteristics

The General Population Trial subject characteristics are shown in Table 6. The median age of participants in this trial at the start of intervention in 1986 was 52 years, slightly

TABLE 6. General Population Trial characteristics

Participant characteristics	All participants	Range for individual treatment factors
No. of participants	29,584	3692–3709
Age (at start of intervention) (y)		
< 50	42%	42%
50–59	35%	34–35%
60+	23%	23–24%
Sex		
Male	45%	44–45%
Female	55%	55–56%
Education		
None	40%	39–41%
Any	60%	59–61%
Tobacco (ever smoked cigarettes regularly > 6 mo)		
No	70%	70–71%
Yes	30%	29–30%
Alcohol[a] (any use in past 12 mo)		
No	77%	76–77%
Yes	23%	23–24%
Pickled vegetable (any use in past 12 mo in winter or spring)		
No	91%	90–92%
Yes	9%	8–10%
Moldy food[b] (any use in past 12 mo)		
No	82%	81–83%
Yes	18%	17–19%
Family history of esophageal cancer[c]		
No	71%	70–72%
Yes	29%	28–30%
Family history of stomach cancer[c]		
No	97%	96–97%
Yes	3%	3–4%

[a] Data missing on 104 participants.
[b] Data missing on 112 participants.
[c] Data missing on 108 participants.

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younger than that in the Dysplasia Trial, and the prevalence of a family history of esophageal or stomach cancer was 33%, also slightly less than that in the Dysplasia Trial. There were no statistically significant differences in any of the subject characteristics examined among any of the eight treatment groups.

Pill Delivery and Compliance Assessment

As with the Dysplasia Trial, commune-based village doctors delivered and counted pills for the General Population Trial. Pill delivery (including use of mailers for participants who had moved out of the area), compliance assessment by pill counts, quality control of pill count compliance assessment, and nutritional assessment were the same as in the Dysplasia Trial. A total of 120 participants were selected from among General Population Trial participants for each quarterly nutritional assessment. Overall, 67% of participants selected took part in the nutritional assessment.

As with the Dysplasia Trial, compliance assessed by monthly pill counts was excellent throughout the study. Eighty-six percent of all participants took over 90% of their pills (range: 85 to 87% across groups by treatment factor) while just 5% were poor compliers (i.e., < 50% of pills taken) (range: 5 to 6% across groups by treatment factor). The overall pill disappearance rate was 93% for all participants with no difference by treatment group (range: 92 to 93%) and little change during the trial (range: 92 to 93% in year 1; 91 to 92% in year 5).

Compliance assessed biochemically over the 5-year intervention is shown in Table 7. Although there were no differences at baseline in retinol, riboflavin, and beta carotene levels, ascorbate levels were significantly lower in the group that was subsequently randomized to receive ascorbic acid. Biochemical assessments during intervention all showed significant differences between individuals who received the factor compared to those who did not. As with the Dysplasia Trial, the proportional increase in beta carotene levels was greater than for the other nutrients assessed. Compared to baseline, values for all four measured nutrients improved during the intervention, evidence for improved general nutrition in the Linxian population during the trial.

TABLE 7. General Population Trial compliance assessed biochemically over 5-year intervention

	Biochemical assessment				
	Baseline[a]		During intervention		
Factor	No.	Mean (SD)	No.	Mean (SD)	P value[b]
		Retinol (μg/dL, plasma)			
A	47	35.7 (8.8)	479	54.0 (16.0)	0.0001
No A	60	35.5 (13.1)	419	43.0 (14.9)	
		Riboflavin (EGR activation coefficient)			
B	56	1.73 (0.34)	747	1.19 (0.25)	0.0001
No B	51	1.78 (0.40)	745	1.44 (0.31)	
		Ascorbic acid (mg/dL, plasma)			
C	49	0.15 (0.13)	730	0.81 (0.47)	0.0001
No C	49	0.25 (0.29)[c]	740	0.54 (0.41)	
		Beta carotene (μg/dL, plasma)			
D	47	5.9 (5.5)	443	85.5 (78.5)	0.0001
No D	60	6.8 (5.8)	455	12.0 (15.0)	

[a] Baseline nutritional assessment, conducted May 1985; values were adjusted for season.
[b] P values are for t tests of factor versus no factor during intervention.
[c] P value for C versus no C at baseline = 0.03.
EGR = erythrocyte glutathione reductase.

End Point Ascertainment

Identification of incident cancers and deaths, retrieval of medical records and diagnostic materials, and expert committee review of cases were performed as in the Dysplasia Trial.

For the General Population Trial, a total of 792 cancer deaths occurred, including 360 due to esophageal cancer and 331 due to stomach cancer (253 from the gastric cardia). A total of 1298 incident cancers were identified, including 639 from the esophagus and 539 from the stomach (including 435 from the gastric cardia). The IERC reviewed 1102 (85%) of these cancers. Of these 1102 cases, the diagnosis was based on histology in 58%, cytology in 52%, and x-ray films in 62%. Only three IERC cases were based on clinical impression without other diagnostic materials.

Statistical Considerations

During the 5 1/4-year intervention, the observed cumulative esophageal/gastric cardia cancer mortality was 2.1% and the observed cumulative esophageal/gastric cancer incidence was 3.6%. With 14,792 participants in each arm and a two-sided test level of 0.05, power was 90% to detect a relative reduction of 26% in esophageal/gastric cardia cancer mortality for each factor tested, and a relative reduction of 19% in incidence.

The primary methods of analysis used included calculation of rates, simple contingency tables, and Poisson regression models which incorporated variables stratified on at randomization and other potential risk factors measured at baseline. A lag period of 12 months following intervention was incorporated in some analyses. Stopping rules and data monitoring were as in the Dysplasia Trial.

ISSUES RELATED TO THE INTERPRETATION OF FINDINGS

There are a number of issues in the interpretation of findings that should be mentioned, including selection of dose and combination of micronutrients, duration of intervention, and the generalizability of results from this population.

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Dosages for the two trials were selected to simultaneously correct deficiencies and maximize potential preventive effects without resulting in toxicity, other adverse side effects, or antagonistic competition between multiple nutrients. Assuming a linear dose-response relation, dosages of target micronutrients one to three times the US recommended daily allowances appeared to achieve all of these objectives.

At the time the trials were being planned, there were ten different micronutrients for which there was both some suspicion of deficiency among the Linxian population and some laboratory evidence of a protective role in experimental animals. Since two trials were anticipated, different design options were possible. With such a large number of micronutrients with potential benefit and the limited size of the population with esophageal dysplasia, the multiple-vitamin/mineral or "cocktail" approach was adopted as the most direct test of the hypothesis in this smaller group of particularly high-risk subjects. The much larger General Population Trial afforded the opportunity to test separate treatment factors although even here there were too many candidate micronutrients to test individually. Overall, the use of two different designs was complementary. Since chemoprevention of cancer was in its infancy at the time, we considered that any significant reduction in cancer would be important and that future studies could define the role of individual factors.

Both scientific and logistical factors were considered when planning how long to run the trials. Lag time to effect was essentially unknown, but typically was estimated to be less than 1 year. Assuming a lag time of 0 to 1 year and the disease rates observed in the population, trials of approximately 5 years' duration provided adequate power to detect reasonable differences. Five-year trials also seemed logistically feasible, while longer interventions increased expense and risked burnout by participants and investigators. In retrospect, our assumptions about lag time may have been too simplistic. It may have been unrealistic to expect a micronutrient intervention to have an impact within 5 years on such a long-term biologic process as carcinogenesis, particularly in a population that has had inadequate nutrition for a lifetime. Longer lag time assumptions, however, have profound implications for study duration. If no benefit is observed in these trials, one potential explanation will be inadequate duration of intervention. Any observed benefits are likely to be minimal estimates, with a greater benefit probable had the interventions continued for longer periods.

Esophageal/gastric cardia cancer in Linxian almost certainly has a multifactorial etiology in which nutrition, other environmental factors and genetic predisposition all have important roles. While results from these trials, positive or negative, will advance our understanding of nutrition and cancer in general, and esophageal carcinogenesis in particular, unique characteristics of the population of Linxian such as their inadequate nutrition and high-risk status may limit the generalizability of these findings to other populations. Only when we more fully understand the role of specific micronutrients and other environmental factors in esophageal carcinogenesis and elucidate predisposition at the molecular genetic level will we know how appropriate it is to generalize these results.

SUMMARY

Although randomized intervention studies using chemopreventive agents are a relatively new approach to the prevention of cancer, they are the most expeditious way to gain specific and definitive information on the relation of specific nutrients to cancer risk. The two nutrition intervention trials described here have a number of unique features in this regard. They are the largest chemoprevention trials initiated to date to evaluate cancer at any site. They were conducted in a distinctive setting—an area of rural China where the rates of esophageal cancer are among the highest in the world, where diet is suspected of playing an important etiologic role, and where the societal structure was particularly logistically conducive to carrying out this daunting effort. The General Population Trial used a fractional factorial design which has not been previously employed in clinical trials, while the Dysplasia Trial used a two-group design. Compliance with the intervention regimens was exceptional, and essentially complete ascertainment of mortality and cancer incidence was achieved. Finally, several special surveys were conducted as part of these trials to examine a variety of biomarkers or intermediate end points of cancer, in further efforts to elucidate the determinants and mechanisms of esophageal carcinogenesis.

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APPENDIX

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3. Data Safety and Monitoring Committee–Paul Engstrom, MD, Chairman, Pelayo Correa, MD, and Stephen Lagakos, PhD.

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